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Effect of foliar nutrition with humic, fulvic acid and proline and their interaction between them on the vegetative growth characteristics of sunflower *Helianthus annuus* L.

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A field experiment was carried out in one of the agricultural fields belonging to Al-Khalis district / Diyala governorate, which is located 14 km north of Baquba district, during the autumn season of 2022, in soil with clay loam texture in order to study the effect of organic fertilization with humic and fulvic acid and spraying the amino acid proline on vegetative groswth. for sunflower plants (*Helianthus annuus* L.) A factorial experiment was carried out with two factors according to the randomized complete block design (RCBD) and with three replications. The first factor was sprayed with humic acid and fulvic acid at concentrations of 6 gm.L.$^{-1}$ For each of them as well as the control treatment, the second agent was sprayed with proline acid at four levels: 0, 10, 20, and 30 mg.L^{-1} The results showed that there was a significant superiority when adding humic acid + fulvic acid at a concentration of 6g.L^{-1} In the average characteristic of plant height, stem diameter, plant dry weight, chlorophyll index, leaf area of the plant, and the number of leaves per plant, which were 160.60 cm, 21.57 mm, 174.25 g, and 57.43 SPAD and 687.31 cm^2 and 38,81 leaves. Plant^{-1}, respectively. The results showed that there was a significant superiority when spraying with proline at a concentration of 30 mg.L^{-1} In the mean of plant height, stem diameter, plant dry weight, chlorophyll index, plant leaf area, and number of leaves per plant, which were 166.95 cm, 21.10 mm, 180.00 g, 54.07 SPAD, and 704.01 cm.^{2} and 40,05 leaves. Plant^{-1} respectively, the results showed that the interaction between the levels of foliar fertilization with humic and fulvic acids and foliar fertilization with proline acid had a significant effect on all traits, as the overlap treatment gave 6 ml.L^{-1} Humic + Fulvic X30 mg.L^{-1} Proline has the highest mean of plant height, stem diameter, plant dryness, chlorophyll index, leaf area of the plant, and number of leaves per plant, which were 171.80 cm, 23.76 mm, 197.00 g, 64.23 SPAD, and 802.82 cm.^{2} And 41,80 leaves^{-1} respectively.

Keywords: Humic acid, fulvic acid, proline acid, organic acids, foliar nutrition, sunflower.

INTRODUCTION

The sun flower plant (*Helianthus annuus* L.) is one of the Compsitae or Asteraceae family plants, grown in subtropical and tropical regions with a dry to semi-arid climate, and its production is affected by drought in the stages of grain filling and flowering (Fatemi *et al.*, 2022), and the plant is grown all over the world because it contains a high oil content (30-50%range?) and its ability To adapt to climatic conditions and different soils, and the plant has many benefits and medical importance as it reduces high cholesterol and blood pressure, as well as it is rich in antioxidants and important vitamins that protect the cell from damage, and it contains essential minerals such as magnesium, zinc and copper and it is important in strengthening the immune system for humans, and its grain meal is a good fodder for farm animals (Mahalik, 2022; Rajeshwaran *et al.*, 2022).

Humic substances are natural organic compounds obtained from decomposing plants, animals, soil, and organic manure. Humic acid is one of the components of humic substances. It stimulates plant growth as well as enhancing the absorption of nutrients as it affects the activity of enzymes, protein, photosynthesis, respiration, and absorption of nutrients. water, nutrients, Cell membrane permeability, Components of Electron Chain Transport, and Free Radical Activity (El-Tahlawy and Ali, 2022). Fulvic acid, the second most important humic substance, is one of the main biostimulants that improve plant production (Capstaff *et al.*, 2020), as a biostimulant, as it attracts water molecules and facilitates the movement of nutrients into the roots, and acts as a mineral

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chelator (Wang and Liu, 2020). Amino acids are among the important compounds that enter into plant growth, as they enter into protein synthesis through the formation of organic nitrogenous compounds as well as the synthesis of alkaloids, dyes and enzymes, and also work to activate cell growth, and store acidity in plant cells because they contain basic aggregates and acidic, and have their role in protecting cells from the high percentage of ammonia, which causes toxicity (Abd-Elkalar *et al.,* 2020), and proline is one of the amino acids involved in the manufacture of protein, and it is one of the most important amino acids for plants because of its accumulation in plant tissues and has an important role in regulating The osmosis process that occurs in the cell because it is concentrated in the cytoplasm and works on the balance of cellular osmosis, and works to protect all enzymes in conditions of water and salt stress, It is a source of nitrogen and carbon, and it has an important role in the flowering and development of plants as the production of proline in plant cells maintains cellular homeostasis, water absorption, osmotic modification and redox balance to restore cell growth and mitigate oxidative damage (Ghosh *et al.,*2022). Therefore, this study aims to:

A study of the spray effect with humic, fulvic acid and proline on the vegetative growth properties of sunflower plants. Determination of the best combination of humic acid, fulvic acid and proline in the vegetative growth of sunflower plants

MATERIALS AND METHODS

A field experiment was carried out during the fall season of 2022 in one of the agricultural fields of Khalis district in Diyala Governorate. A factorial experiment was carried out according to a randomized complete block design (RCBD) with three replicates in clay soil. Table 1 shows some of the physical and chemical characteristics of study soil, which was conducted in the laboratory of the Directorate of Agriculture of Diyala. Random samples were taken from different areas of the field soil from a depth of 0-30 cm for the purpose of laboratory analysis before the cultivation and fertilization process. These samples were mixed well for the purpose of homogenization and dried air. Then it was ground, smoothed, and passed through a sieve with a diameter of 2 mm, and a representative sample was taken from it for the purpose of conducting an analysis of some physical and chemical characteristics, which was conducted in the laboratory of the Directorate of Agriculture of Diyala, as shown in Table 1. The experiment included two factors with three replications. The first factor was foliar feeding with humic and fulvic acid at a concentration of 6g.L.$^{-1}$ For each of them, there were four treatments: humic acid foliar spray on vegetative total, foliar spraying of fulvic acid on vegetative total, humic + fulvic acid spraying and spraying with distilled water only as a control treatment. The second factor included proline acid foliar spraying on vegetative total with four concentrations of 0, 10, 20 and 30 mg.Liter^{-1}, the number of transactions has become 16 treatments with three repeated to become 48 experimental units. The experimental treatments were sprayed on the shoots until complete wetness and in the early morning to avoid high temperatures. The area of the experimental unit was 2m^2, with four lines in one experimental unit, the distance between one pit and another is 25 cm, the distance between one line and another is 60 cm, the distance between experimental units is 1 m, and between replicates is also 1 m, with aplant density of 66,666 plants.ha^{-1} is it ha? The seeds were sown in the field on month of july 2022manually, with three seeds in one pit (5cm^2). Sunflower seeds of the Luleo variety were used. The fungicide (Proplant) was added at a concentration of 72.2% three times throughout the growing season, and the insecticide (Cyprin) was also added. Once to combat the whitefly. Vegetative plant traits were measured.

Table 1. Some physical and chemical properties of agricultural soil.

analysis type	the value	Unit
Soil salinity Ec	3.8	dsm^{-1}
Soil acidity pH	7.3	--
Soil texture	clay loam	
Clay	384.8	gm. kg^{-1}
Silt	343	gm. kg^{-1}
Sand	272.2	gm. kg^{-1}

***Studied traits*:** Plant characteristics were calculated for the average of five plants randomly taken from the two middle lines of each experimental unit.

1. Characteristics of vegetation

***plant height(cm)*:** It was measured by a measuring tape from the plant connection area with soil to the base of the flower disc for five plants from the two middle lines, then an average was taken (Al-Hamdani and Suleiman, 2014).

***The number of leaves (leaf plant^{-1})*:** The number of total plant leaves at the flowering stage was calculated for five plants from each experimental unit, and then an average was calculated.

***Stem diameter (mm)*:** Five plants were selected from each experimental unit, and the stem diameter was measured at the flowering stage from the middle of the stem by the foot (Vernier), then the average was calculated.

***leaf area (cm^2)*:** The leaf area was calculated at the flowering stage by calculating the sum of the squares of the maximum width of the leaves according to the mentioned method LA=$\sum$ W^{2}0.65 (Elsahookie and Eldabas 1982).

***Leaf Chlorophyll Index (SPAD)*:** The chlorophyll index in the leaves was measured using the SPAD-502 device.

***Statistical Analysis*:** The data were analyzed statistically using the statistical analysis program (Genstat) according to the analysis of variance (SAS) as an experiment within the styling of the full random sectors, the medians of the treatments were the compared according to Duncan multiple

test at the level of probability 0.05% (Al-Rawi and Khalafallah, 2000).

RESULTS AND DISCUSSION

***Plant height(cm)*:** The results in Table 2 indicate that there are significant differences in the average height of sunflower plants when organic fertilizers were added, and the highest average of plant height was when spraying with humic acid and fulvic acid at a concentration of 6 g. Liter^{-1} Which amounted to 160.60 cm compared to non-additive treatment, It reached 147.98 cm, with an rate of 8.52%. The reason may be attributed to the fact that spraying humic and fulvic acid increases the uptake of monovalent ions such as ammonium and potassium by accelerating the active uptake by plant roots (Taguddin and Barakat, 2017).The results also showed that there were significant differences in plant height when adding proline acid, as it gave the treatment of adding proline at a concentration of 30 mg. Liter^{-1} The highest average plant height was 166.95 cm, compared to the non-additive treatment, which reached 141.25 cm, with an increase of 18.19%. The reason is attributed to the fact that spraying proline led to improved plant growth and increased height, since proline is a source of nitrogen, as it contributes to building protein, which has an important role in supplying plants with the energy necessary for growth and construction processes, and thus increasing plant height (Abass, 2015; Al-Dami, 2017). The results showed that there were significant differences when the addition of organic fertilizers interfered with proline acid, and the highest mean of plant height was when the interaction treatment added organic fertilizers at a concentration of 6 g. L^{-1} And proline acid at a concentration of 30 mg. Liter^{-1} It was 171.80 cm, compared to the control treatment, which was 134.13 cm, with an increase of 28.08%. ***leaves number (leaf plant^{-1})*:** The score in Table 3 indicated that are significant differences in average number of leaves of the sunflower plant when adding organic fertilizers, as the highest average number of leaves was when adding the treatment humic acid and fulvic acid at a concentration of 6 g. L^{-1} Which amounted to 38.81 leaf. plants^{-1} Compared to the treatment of no addition, which amounted to 33.68 leaves. plant^{-1} And an increase rate of 15.23%, the reason may be attributed to the role of humic and fulvic acid in causing an increase in the rate of leaves, due to their effect on stimulating

Table 2. Effect of foliar feeding with humic, fulvic and proline and interaction between them on average hikes of sunflower plant (cm).

Proline acid	Proline acid concentrations mg. Liter^{-1}				
Organic fertilization	0	10	20	30	Average effect of organic fertilization
without addition	134.13	146.06	152.40	159.33	147.98
	i	fg	It is	cd	C
humic acid	140.60	152.33	163.86	168.46	156.31
6 gm. Liter^{-1}	h	e	bcd	ab	B
fulvic acid	142.66	150.06	161.33	168.20	155.56
6 gm. Liter^{-1}	gh	fe	cd	ab	B
Humic + fulvic acid 6	147.60	158.40	164.60	171.80	160.60
gm. Liter^{-1}	fg	d	bc	a	A
average effect of	141.25	151.71	160.55	166.95	
proline	d	c	b	a	

Values with similar letters, there are no significant differences between them

Table 3. Effect of foliar feeding with humic acid, fulvic acid and proline and the interaction between them on the average number of leaves of the sunflower plant (leaf. plant^{-1}).

Proline acid	Proline acid concentrations mg. Liter^{-1}				
Organic fertilization	0	10	20	30	Average effect of organic fertilization
without addition	30.53	32.06	35.13	37.00	33.68
	j	ij	fgh	cdefg	B
humic acid	33.66	36.06	39.53	40.80	37.51
6 gm. Liter^{-1}	hi	efgh	abcd	ab	A
fulvic acid	34.20	36.46	38.80	40.60	37.51
6 gm. Liter^{-1}	take note	defgh	abcde	ab	A
Humic + fulvic acid 6	35.66	38.06	39.73	41.80	38.81
gm. Liter^{-1}	efgh	bcdef	abc	a	A
average effect of	33.51	35.66	38.30	40.05	
proline	d	c	b	a	

Values with similar letters, there are no significant differences between them

the vital processes in plant tissues such as the biosynthesis of photosynthesis products in the leaves and encouraging cell division and increasing their number, which leads to building the vegetative system and increasing the number of leaves (Al-Bayati and Khalifa, 2019).

The results also showed that there were significant differences in the number of leaves when adding proline acid, as the treatment gave the addition of proline at a concentration of 30 mg.L^{-1} The highest average number of leaves was 40.05 (leaves.$plant^{-1}$) Compared to the non-addition treatment, amounted 33.51 ($leaves^{-1}$ .plant)an increase of 19.51%, the causes may be attributed to the fact that proline works as an osmosis regulator that controls the osmotic pressure in the plant to absorb water and play a key and effective role in many vital processes of the plant and maintains the chloroplast membranes and increases the efficiency of photosynthesis (El-Nwehy, 2022). The results showed that there were significant differences when the addition of organic fertilizers interfered with proline acid, and the highest average leaves number was when treatment of interaction spray the organic fertilizers at a concentration of 6 g.L^{-1} And proline acid at a concentration of 30 mg. $Liter^{-1}$ It amounted to 41.80 (leaves. $Plant^{-1}$) Compared to control treatment, amounted 30.53 (leaves.$plant^{-1}$) an increase of 36.91%.

***Stem diameter (mm)*:** The score in Table 4 indicate that are significant differences in stem diameter of sunflower plants when organic fertilizers are added, and the highest average stem diameter was when spraying with humic acid and fulvic acid at a concentration of 6 g. L^{-1} which amounted to 21.57 mm, compared to the non-addition treatment, which amounted to 17.32 mm, with an increase rate of 24.53%.The cause of the increase in the role of fulvic and humic acid in promoting multiple functions such as increasing the permeability of the cell membrane, raising the photosynthesis efficiency of plants, and controlling hormone levels *(*Islam *et al*., 2020).

The results also showed that there were significant differences in the diameter of the stem when adding proline acid, as it gave the treatment of adding proline at a concentration of 30 mg. $Liter^{-1}$ The highest average height of the plant was 21.10 mm compared to the treatment without addition, which reached 17.67 mm, with an increase rate of 19.41%. The reason is attributed to the spraying of Proline led to a decrease in the osmotic potential, and in turn reduced the water

Table 4. Effect of foliar feeding with humic, fulvic and proline and the interaction between them on the average stem diameter of sunflower plants (mm).

Proline acid	Proline acid concentrations mg. $Liter^{-1}$				
Organic fertilization	**0**	**10**	**20**	**30**	**Average effect of organic fertilization**
without addition	15.10 h	16.33 g	18.20 f	19.66 of	17.32 c
humic acid 6 gm. $Liter^{-1}$	17.36 f	19.80 cde	19.40 It is	20.60 bcd	19.29 b
fulvic acid 6 gm. $Liter^{-1}$	18.10 f	19.66 of	19.53 of	20.36 cde	19.41 b
Humic + fulvic acid 6 gm. $Liter^{-1}$	20.13 cde	21.56 b	20.83 bc	23.76 a	21.57 a
average effect of proline	17.67 c	19.34 b	19.49 b	21.10 a	

Values with similar letters, there are no significant differences between them

Table 5. Effect of foliar feeding with humic acid, fulvic acid and proline and the interaction between them on the average leaf area of sunflower plant $(cm)^2$).

Proline acid	Proline acid concentrations mg. $Liter^{-1}$				
Organic fertilization	**0**	**10**	**20**	**30**	**Average effect of organic fertilization**
without addition	481.70 i	522.86 h	540.32 gh	607.87 of	538.19 d
humic acid 6 gm. $Liter^{-1}$	556.13 gh	597.84 def	682.36 bc	716.41 b	638.19 c
fulvic acid 6 gm. $Liter^{-1}$	538.27 gh	573.13 efg	618.68 d	688.94 bc	604.76 b
Humic + fulvic acid 6 gm. $Liter^{-1}$	566.61 fg	667.23 c	712.60 b	802.82 a	687.31 a
average effect of proline	535.68 d	590.26 c	638.74 b	704.01 a	

Values with similar letters, there are no significant differences between them

potential of the cell and thus increased its ability to withdraw water and dissolved nutrients from the growth medium. And then increase the growth of cells in the plant and protect the cell from oxidative stress as it is a catcher of oxidative free radicals and thus increase the diameter of the stem (Kaur and Asthir, 2015).

The results showed that there were significant differences when the addition of organic fertilizers interfered with proline acid, and the highest mean of the diameter of the stem was when the treatment of overlapping the addition of organic fertilizers at a concentration of 6 g. L^{-1} And proline acid at a concentration of 30 mg. L^{-1} It was 23.76 mm compared to the control treatment, which was 15.10 mm, with an increase of 57.35%.

***Leaf area (cm^2)*:** The results in Table 5 indicate that there are significant differences in the average leaf area of sunflower plants when adding organic fertilizers, and the highest average leaf area was when adding humic acid and fulvic acid at a concentration of 6 g. $Liter^{-1}$ which amounted to 687.31 cm^2 Compared with the non-addition treatment, which amounted to 538.19 cm^2 With an increase rate of 27.70%, the reason may be attributed to the role of humic and fulvic acid in increasing the amount of major and micronutrients ready for absorption. Vegetative growth and increase the effectiveness of meristems, and then increase the leaf area of the plant (Al-Tamimi and Al-Jadri, 2017).

The results also showed that there were significant differences in the average leaf area when adding proline acid, as it gave the treatment of adding proline at a concentration of 30 mg. L^{-1} The highest average leaf area was 704.01 cm^2 Compared to the non-addition treatment, which amounted to 535.68 cm^2 With an increase of 31.42%, the causes for this superiority to the positive role in regulating the osmotic potential, which increases the ability of the cell to withdraw water from the growth medium, and then increase plant growth, cell elongation, perpetuating the opening and closing of stomata and the process of photosynthesis, in addition to the fact that proline acid is a source Nitrogen contributes to building protein and has a role in supplying the plant with energy, leading to an increase in leaf area (Al-Ghanmi *et al.*, 2015).

The results showed that there were significant differences when the addition of organic fertilizers interfered with proline acid, and the highest mean of the leaf area was when the interaction treatment added organic fertilizers at a concentration of 6 g. L^{-1} And proline acid at a concentration of 30 mg. L^{-1} It was 802.82 cm $long^2$ Compared to the control treatment, which amounted to 481.70 cm^2 with an increase of 66.66%.

***Leaf Chlorophyll Evidence (SPAD)*:** The results in Table 6 indicate that are significant differences in the mean chlorophyll index of sunflower plants when adding organic fertilizers, and the highest mean of the chlorophyll index was when adding humic acid and fulvic acid at a concentration of 6 g. L^{-1} Which amounted to 57.43 SPAD compared to the treatment of no addition, which amounted to 44.85 SPAD, and an increase rate of 28.04%. The reason is attributed to the direct role of humic acid in increasing the antioxidants and maintaining the chlorophyll content in the leaves from the catabolism process, and also has an indirect effect on the soil by increasing the root system. Thus, it encourages the absorption of nitrogen and magnesium from the soil, which increase the content of leaves of chlorophyll, (Shah *et al.*, 2018; Faizy, 2019).

The results also showed that there were significant differences in the index of chlorophyll when adding proline acid, as it gave the treatment of adding proline at a concentration of 30 mg. L^{-1} The highest average index of chlorophyll was 54.07 SPAD compared to the treatment of no addition, which amounted to 48.02 SPAD, with an increase of 12.59%. The reason for this increase may be due to the fact that spraying the amino acid stimulated the construction of chlorophyll pigments and the formation of plastid granules, and thus increased the chlorophyll content in the leaves. It is the regulatory function of proline in the detoxification of free radicals under salinity stress, which causes the oxidation of lipids in the cell membrane (El-Nwehy, 2022).

Table 6. Effect of foliar feeding with humic, fulvic acid and proline and the interaction between them on average chlorophyll index of sunflower plants (SPAD).

Proline acid	**Proline acid concentrations mg. $Liter^{-1}$**				
Organic fertilization	**0**	**10**	**20**	**30**	**Average effect of organic fertilization**
without addition	43.36 f	44.05 f	44.36 f	47.62 def	44.85 d
humic acid 6 gm. $Liter^{-1}$	46.87 if	48.52 def	49.14 def	51.26 cde	48.95 c
fulvic acid 6 gm. $Liter^{-1}$	50.70 cde	51.87 cde	53.51 bcd	53.42 bcd	52.37 b
Humic + fulvic acid 6 gm. $Liter^{-1}$	51.13 cde	55.48 bc	59.11 ab	64.23 a	57.43 a
average effect of proline	48.02 c	49.98 bc	51.53 ab	54.07 a	

Values with similar letters, there are no significant differences between them

Table 7. Effect of foliar feeding with humic, fulvic and proline and interaction between them on average dry weight to sunflower plant (gm).

Proline acid	Proline acid concentrations mg. Liter^{-1}				
Organic fertilization	**0**	**10**	**20**	**30**	**Average effect of organic fertilization**
without addition	125.66 h	140.00 gh	146.33 fg	151.33 efg	140.83 c
humic acid 6 gm. Liter^{-1}	137.00 gh	149.33 efg	164.00 cde	193.00 ab	160.83 b
fulvic acid 6 gm. Liter^{-1}	138.00 gh	147.00 fg	162.00 def	178.66 bc	156.41 b
Humic + fulvic acid 6 gm. Liter^{-1}	153.66 efg	170.00 cd	176.33 cd	197.00 a	174.25 a
average effect of proline	138.58 d	151.58 c	162.16 b	180.00 a	

Values with similar letters, there are no significant differences between them

The results showed that there were significant differences when the addition of organic fertilizers interfered with proline acid, and the highest average of the index of chlorophyll was when the interaction treatment added organic fertilizers at a concentration of 6 g. L^{-1} And proline acid at a concentration of 30 mg. L^{-1} It reached 64.23 SPAD, compared to the control treatment, which amounted to 43.36 SPAD, with an increase rate of 48.13%.

***Plant dry weight (g)*:** The score in Table 7 indicate that are significant differences in average dry weight of sunflower plants when adding organic fertilizers, and the highest average dry weight was when adding humic acid and fulvic acid at a concentration of 6 g. L^{-1} Which amounted to 174.25 gm compared to the treatment of no addition, which amounted to 140.83 gm, and an increase rate of 23.73%. The causes for this superiority due to role of humic and fulvic acid, as they work to increase the permeability of cell membranes and the transport of elements inside the plant, which contributed to an increase in cell size, elongation and division. The reason for an increase in vegetative growth indicators (Al-Khalidi *et al.*, 2021).

The results showed that there were no significant differences in the average dry weight of the plant when treated with humic acid and the treatment with fulvic acid, as the average dry weight of the plant was 160.83 and 156.41 gm, respectively.

The results also showed that there were significant differences in the average dry weight when adding proline acid, as the treatment gave the addition of proline at a concentration of 30 mg. L^{-1} The highest average dry weight was 180.00 gm compared to the no-additive treatment, amounted 138.58 gm, with an increase of 29.88%. The causes due to important role of the proline, as it works to reduce the percentage and concentration of salinity to which plants in the soil are exposed due to its ability to absorb large quantities. of water and increase biomass (plant height and leaf area, Tables 2 and 5) and thus increases tolerance to salt stress, which leads to an increase in plant dry weight (Al-Hassani *et al.*,2019).

The results showed that there were significant differences when the addition of organic fertilizers interfered with proline acid, and the highest average of dry weight was when the interaction treatment added organic fertilizers at a concentration of 6 g. L^{-1} And proline acid at a concentration of 30 mg. L^{-1} It reached 197.00 gm compared to control treatment, amounted to 125.66 gm, the rate of increase 56.77%.

***Conclusion*:** The effect of folia nutrition with humic and fulvic acid at a contcentration of 6mg.L^{-1} with a concentration of 30mlg.L^{-1} of the amino acid proline had a significant effect on all studied triats. The effect of folia nutrition with humic, fulvic acid and proline had a significant effect on all studied triats. Conclusion should be very brief and clear

***Authors' contributions*:** Baidaa Majeed Rashid Awaid, Run the analyses and wrote the article, and cooperate with Najm Abdullah Juma Al-Zubaidi in conceiving the idea and designing the study, Najm Abdullah Juma Al-Zubaidi A supervised all parts of the manuscript.

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***Availability of data and material*:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

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